

Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation

Mee, Jessica; Gibson, Oliver; Watt, Peter; Doust, Jo; Maxwell, Neil; Taylor, Lee; Tuttle, James

Temperature

DOI:

[10.1080/23328940.2016.1214336](https://doi.org/10.1080/23328940.2016.1214336)

Published: 01/01/2016

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Mee, J., Gibson, O., Watt, P., Doust, J., Maxwell, N., Taylor, L., & Tuttle, J. (2016). Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation. *Temperature*, 3(4), 549-556. <https://doi.org/10.1080/23328940.2016.1214336>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Title

Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation

Authors

Mee J.A.^{1, 2}, Gibson O.R.^{1, 3}, Tuttle J.A.⁴, Taylor L.^{5, 6}, Watt P.W.¹, Doust J.¹, and Maxwell N.S.¹

¹Centre for Sport and Exercise Science and medicine (SESAME). Environmental Extremes Laboratory, University of Brighton, Welkin Human Performance Laboratories, Denton Road, Eastbourne, UK.

² School of Sport, Health, and Exercise Science, Bangor University, Bangor, LL57 2PZ

³ Centre for Human Performance, Exercise and Rehabilitation (CHPER), Brunel University, London, UK

⁴ Muscle Cellular and Molecular Physiology (MCMP) and Applied Sport and Exercise Science (ASEP) Research Groups, Institute of Sport and Physical Activity Research (ISPAR), University of Bedfordshire, Bedford, UK.

⁵ ASPETAR, Qatar Orthopaedic and Sports Medicine Hospital, Athlete Health and Performance Research Centre, Aspire Zone, Doha, Qatar.

⁶ School of Sport, Exercise and Health Sciences. Loughborough University, Loughborough, UK.

Corresponding Author

Jessica A. Mee J.A.Mee@bangor.ac.uk

College of Health and Behavioural Sciences, Bangor University, Bangor, LL57 2PZ

Tel +44 (0) 1248 388309

Acknowledgements

The author would like to thank the volunteers for their participation in this investigation. Funding and support was obtained from the University of Brighton and the University of Bedfordshire.

Conflict of interest

The authors of this study declare they have no conflict of interest

List of abbreviations

BM	Body mass
CT	Cycle threshold
HA	Heat acclimation
HR	Heart rate
Hsp72 mRNA	Heat shock protein 72 messenger ribonucleic acid
HSP	Heat shock protein
HSR	Heat shock response
RH	Relative humidity
RT- QPCR	Reverse transcription polymerase chain reaction
T _{re}	Rectal temperature

Abstract

Purpose: Thermotolerance is an acquired state of increased cytoprotection achieved following single or repeated exposures to heat stress, in part characterised by changes in the intracellular 72kda heat shock protein (HSP72; HSPA1A). Females have demonstrated reduced exercise induced HSP72 in comparison to males. This study examined sex differences in heat shock protein 72 messenger ribonucleic acid (Hsp72 mRNA) transcription during heat acclimation (HA) to identify whether sex differences were a result of differential gene transcription.

Methods: Ten participants (5M, 5F) performed ten, 90 min controlled hyperthermia [rectal temperature (T_{re}) $\geq 38.5^{\circ}\text{C}$] HA sessions over 12 d. Leukocyte Hsp72 mRNA was measured pre and post D1, D5, and D10, via Reverse transcription polymerase chain reaction (RT-QPCR).

Results: HA was evidenced by a reduction in resting T_{re} ($-0.4 \pm 0.5^{\circ}\text{C}$) and resting heart rate [(HR); $-13 \pm 7 \text{ beats}\cdot\text{min}^{-1}$] following HA ($p \leq 0.05$). During HA no difference ($p > 0.05$) was observed in ΔT_{re} between males (D1 = $1.5 \pm 0.2^{\circ}\text{C}$; D5 = $1.6 \pm 0.4^{\circ}\text{C}$; D10 = $1.8 \pm 0.3^{\circ}\text{C}$) and females (D1 = $1.5 \pm 0.5^{\circ}\text{C}$; D5 = $1.4 \pm 0.2^{\circ}\text{C}$; D10 = $1.8 \pm 0.3^{\circ}\text{C}$). This was also true of mean T_{re} demonstrating equality of thermal stimuli for mRNA transcription and HA. There were no differences ($p > 0.05$) in Hsp72 mRNA expression between HA sessions or between males (D1 = $+1.8 \pm 1.5$ fold; D5 = $+2.0 \pm 1.0$ fold; D10 = $+1.1 \pm 0.4$ fold) and females (D1 = $+2.6 \pm 1.8$ fold; D5 = $+1.8 \pm 1.4$ fold; D10 = $+0.9 \pm 1.9$ fold).

Conclusions: This experiment demonstrates that there is no difference in Hsp72 mRNA increases during HA between sexes when controlled hyperthermia HA is utilised. Gender specific differences in exercise-induced HSP72 reported elsewhere likely result from post-transcriptional events.

Key words:

Controlled hyperthermia; males; females; thermotolerance; heat shock protein.

81 Introduction

82 Repeated exposure to stressful thermal environments initiates heat adaptation in humans [1]. Heat
83 adaptation incorporates the interrelated acclimation and thermotolerance [2,3]. A heat acclimated
84 phenotype describes enhanced heat loss effector responses and hypervolemia which mitigate
85 physiological, perceptual, and functional detriments to heat exposure [1,4]. Thermotolerance or
86 acquired cellular thermotolerance is the nomenclature used to describe cellular adaptations caused
87 by a single, or repeated severe, but non-lethal heat exposure [e.g. heat acclimation (HA)] [5].

88 HA repeatedly initiates the heat shock response (HSR), typically increasing various basal heat shock
89 proteins (HSP), including HSP72 (HSP72). In response to 10 d HA, baseline intracellular HSP72 has been
90 shown to increase by 18% [6] whilst heat shock protein 72 messenger ribonucleic acid (Hsp72
91 mRNA) demonstrates a pattern whereby transcription occurs within each HA session (+195%) before
92 returning to baseline 24 hr later [7,8]. These transient HA mediated cellular adaptations to iHSP72 can
93 confer cytoprotection to subsequent thermal [6] and non-thermal [8] stressors *in vitro* [6] and *in vivo*
94 [9]. Eloquent in-vitro data demonstrates that cytoprotection to stress (thermal or otherwise) is
95 abolished when HSP72 is knocked out [10–12] or blocked [13]. HA mediated in-vivo cytoprotection is
96 dependent upon sufficient Hsp72 mRNA transcription [14] and subsequent HSP72 protein translation
97 [15].

98 Controlled hyperthermia HA results in a greater Hsp72 mRNA compared with matched training in cool
99 conditions as a result of greater endogenous stimuli for transcription [8]. Due to the consistent
100 endogenous thermal stimulus there is an equality of Hsp72 mRNA transcription during 10 d controlled
101 hyperthermia HA [7]. Thus, controlled hyperthermia is a preferred HA method compared with
102 traditional exogenously prescribed HA since it induces robust Hsp72 mRNA responses, ensuring
103 sufficient and consistent increases in endogenous stimuli throughout an *in vivo* chronic intervention,
104 particularly when comparing independent groups.

Morton and colleagues (2009) reported a sex specific HSP adaptation in human skeletal muscle following six weeks of continuous and interval training. Specifically, HSP70 increased by $38 \pm 41\%$ and $23 \pm 36\%$ following continuous and interval training respectively in males ($n = 5$); however females ($n = 5$) had no changes ($3 \pm 37\%$ and $4 \pm 14\%$ increase respectively), despite similar training status, training prescription and training adaptations ($\dot{V}O_2$ max) [16]. Differential sex responses reported by Morton et al. (2009) may be attributed to cytoprotective effects of oestrogen. Elevated oestrogen has been shown to afford cellular protection [17], accordingly increased oestrogen in females versus males may provide a mechanism for inhibited changes in HSP72 expression [18]. Oestrogen binds to the oestrogen receptor, which is a member of the steroid family of nuclear receptors and is the oestrogen response element in target genes, leading to the transcriptional regulation of many genes [19]. Gillum et al. (2013) reported higher intracellular HSP72 concentrations following a single bout of exercise in the heat in males compared with females (in both the follicular and luteal phase of the menstrual cycle), despite similar baseline values and identical endogenous stimuli for Hsp72 mRNA transcription [20]. Differential sex responses were also suggested to be a result of oestrogen providing cellular protection and thus, decreasing the necessity for translation of HSP72 in females. Although, stress-mediated sex specific differences in the HSP72 have been seen [16,20], they have not been examined at an mRNA level across the course of controlled hyperthermia HA.

Determination of Hsp72 mRNA transcription in females would facilitate identification of whether the inhibited HSP72 response resulted from absent gene signalling, or mitigated protein translation, potentially due to elevated oestrogen [16,20]. Absence of data in female populations could be problematic for practitioners who may adopt HA protocols that are informed by mechanistic cellular adaptations from male only cohorts [7,21]. This may reduce the magnitude to which females are protected against heat injury [14].

The aim of the current study was to determine whether the Hsp72 mRNA response during controlled hyperthermia HA, differed between males and females. It was hypothesised that the Hsp72 mRNA

response would be attenuated in females compared to males across the course of controlled hyperthermia HA.

Materials and methods

Participants

Based on *a priori* power analyses using previous experimental data with identical methods [7,8], four participants in each group would result in 95% probability of detecting a difference in Hsp72 mRNA across the course of controlled hyperthermia HA. In line with power analysis, and previous work in the area [16], five males and five females (table 1) provided written informed consent to participate in the current study. All procedures were performed in accordance with the ethical standards of the institute and with the 1964 Declaration of Helsinki, as revised in 2013. Experimental trials were performed between 07:00 and 10:00 h to control for the time of day effects [7,22]. Confounding variables of smoking, caffeine, glutamine, alcohol, generic supplementation, and prior thermal, hypoxic, and hyperbaric exposures were all controlled in line with previous work in the field [23]. To control for hormonal fluctuations associated with the menstrual cycle, female participants began testing during the early follicular phase (3 d after the onset of menstruation) of their self-reported menstrual cycle; where oestrogen ($\sim 30 \text{ pg.ml}^{-1}$) and progesterone ($\sim 1 \text{ ng.ml}^{-1}$) value are expected to be stable.

Table 1 Participant characteristics. Mean \pm SD.

	Age	Height	BM	$\dot{V}\text{O}_2$ peak	
	(years)	(cm)	(kg)	(L.min ⁻¹)	(mL.kg ⁻¹ .min ⁻¹)
Males (N = 5)	24 \pm 7	175 \pm 3	70.1 \pm 5.1	2.64 \pm 0.34	45.7 \pm 4.4
Females (N = 5)	20 \pm 1	163 \pm 9	57.1 \pm 4.9	3.22 \pm 0.50	46.2 \pm 4.1

Notes: BM, body mass; $\dot{V}\text{O}_2$ peak, peak oxygen uptake

Preliminary testing

2 hr prior to arrival participants consumed 3-5 mL.kg⁻¹ of water. On arrival to the laboratory for all experimental sessions, participants voided their bladder to provide a mid-flow urine sample. When two out of the following three criteria were achieved, adequate hydration to perform the trial was assumed based upon an osmolality value of ≤ 700 mOsm.kg⁻¹, a urine specific gravity value of ≤ 1.020 or body mass within 1% of daily average [24]. These experimental controls were not violated for any participant for any of the preliminary or experimental procedures. Height was measured using a fixed stadiometer recorded to 1 cm (Detecto Physicians Scales; Cranlea & Co., Birmingham, UK), and nude body mass (BM) recorded to 0.01 kg from digital scales (ADAM GFK 150, USA). A graded exercise test was performed in temperate laboratory conditions [20°C, 40% relative humidity (RH)] to determine $\dot{V}O_2$ peak using a cycle ergometer (Monark e724, Vansbro, Sweden). The cycling intensity was set to 80 W and resistance was applied to the flywheel to elicit a 16 to 24 W.min⁻¹ increase (selected depending on the BM of the participant). Expired air was measured using online gas analysis (Metalyzer Sport, Cortex, Germany). Peak $\dot{V}O_2$ was considered the highest $\dot{V}O_2$ obtained in any 30 s period.

Experimental design

Two, 5 d consecutive blocks (10 d total) of controlled hyperthermia HA were completed separated by 48 hrs. Immediately prior to each HA session participants inserted a rectal thermometer (Henley, Reading, UK) 10 cm past the anal sphincter and affixed a heart rate (HR) monitor (Polar Electro Oy, Kempele, Finland). After a 20 min seated stabilisation period, resting measures were recorded and participants entered the environmental chamber (TISS, Hampshire, UK). The daily sessions consisted of a 90 min exposure to 40°C, 40% RH. Exercise intensity was set at 65% $\dot{V}O_2$ peak from the outset and adjusted with work: rest intervals to maintain a rectal temperature (T_{re}) $\sim 38.5^\circ\text{C}$ [25,26]. T_{re} and HR were recorded at 5 min intervals. Fluid intake was restricted during the 90 min HA session.

Blood sampling, RNA extraction, and one-step reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Venous blood samples were taken immediately before and immediately after exercise heat exposure on D1, D5, and D10 of controlled hyperthermia HA. All blood samples were drawn from the antecubital vein into 6 mL EDTA Vacuette tubes (Grenier BIO-One, Stonehouse, UK). 1 mL of venous blood was pipetted into 10 mL of 1 in 10 red blood cell lysis solution (10X red blood Cell Lysis Solution; Miltenyi Biotech, Bisley, UK). Samples were incubated for 15 min at room temperature then isolated via centrifugation at 5°C and 400 *g* for 5 min and washed twice in 2 mL phosphate-buffered saline at 400 *g* for 5 min to isolate all leukocytes. RNA was then extracted via the previously validated acid guanidium thiocyanate–phenol–chloroform extraction method [27]. Quantity was determined at an optical density of 260 nm while quality was determined via the 260/280 and 260/230 ratios using a nanodrop spectrophotometer (NanoDrop 2000c; Thermo Scientific, Waltham, MA, USA).

Hsp72-relative mRNA expression (Hsp72 mRNA) was quantified using Reverse transcription polymerase chain reaction (RT-QPCR). Primers (table 2) were designed using primer design software (Primer Quest and Oligoanalyzer; Integrated DNA Technologies, Coralville, IA, USA). 20 µL reactions containing 10 µL SYBR-Green RT-PCR Mastermix (Quantifast SYBRgreen Kit; Qiagen, Manchester, UK), 0.15 µL forward primer, 0.15 µL reverse primer, 0.2 µL reverse transcription mix (Quantifast RT Mix; Qiagen) and 9.5 µL sample (70 ng RNA/µL) were prepared in separate tubes. Each PCR reaction (Rotorgene Q; Qiagen) was then performed as follows: 10 min, 50°C (reverse transcription), 5 min 95°C (transcriptase inactivation and initial denaturation); followed by: 10 s, 95°C (denaturation), 30 s, 60°C (annealing and extension) for 40 cycles. Fluorescence was measured following each cycle as a result of the incorporation of SYBR green dye into the amplified PCR product. Melt curves (50 to 95°C; ramp protocol 5-s stages) were analysed for each reaction to ensure only the single gene of interest was amplified. The relative quantification of mRNA expression for each sample was assessed by determining the ratio between the cycle threshold (CT) value of the target mRNA and the CT values

for β 2-microglobulin. Fold change in relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method [28].

Table 2 Hsp72 mRNA primer sequences.

Gene	NCBI Accession #	Primer	Sequence (5'→3')	Amplitude length
B2- Microglobulin (β2-M)	NM_004048	Forward	CCGTGTGAACCATGTGACT	91
		Reverse	TGCGGCATCTTCAAACCT	
Hsp72	NM_005345	Forward	CGCAACGTGCTCATCTTTGA	198
		Reverse	TCGCTTGTTCTGGCTGATGT	

Notes: NCBI National Centre for Biotechnology Information

Statistical analysis

All data were first checked for normality using the Shapiro-Wilk method and corrected for sphericity using the Greenhouse Geisser method. A two way mixed design analysis of variance (ANOVA) was performed to determine differences between the physiological and performance characteristics between D1, D5, and D10 in males and females. A three-way mixed design ANOVA was performed to identify differences between the Hsp72 mRNA, pre and post, on D1, D5, and D10 of controlled hyperthermia HA between males and females. When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison. Effect sizes [partial eta squared (η^2)] were calculated to analyse the magnitude of trends associated with controlled hyperthermia HA. All data were analysed using a standard statistical package (SPSS version 20.0, IBM, Armonk, New York, USA) and reported as mean \pm SD. Statistical significance was accepted at the level of $p \leq 0.05$.

Results

Evidence of heat acclimation

Figure 1 presents the resting T_{re} and resting HR data for D1, D5, and D10 of controlled hyperthermia HA. There was a main effect of day on T_{re} rest ($F_{(2, 16)} = 11.219$, $p \leq 0.001$, $np^2 = 0.584$). No differences were observed from D1 to D5 ($p = 0.563$). However, T_{re} rest reduced from D1 to D10 ($p = 0.027$) and from D5 to D10 ($p = 0.003$). There was no interaction effect between day and sex on T_{re} rest ($F_{(2, 16)} = 3.287$, $p = 0.064$, $np^2 = 0.291$), however this was approaching significance with a moderate effect. The mean reduction in T_{re} rest from D1 to D5 was $-0.3 \pm 0.2^\circ\text{C}$ in males, whereas in females there were no changes ($+0.1 \pm 0.2^\circ\text{C}$). The mean reduction in T_{re} rest from D5 to D10 was greater in females ($-0.4 \pm 0.2^\circ\text{C}$) compared with males ($-0.1 \pm 0.1^\circ\text{C}$).

There was a main effect of day on HR rest ($F_{(2, 16)} = 15.227$, $p \leq 0.001$, $np^2 = 0.656$). HR rest reduced from D1 to D5 ($p = 0.040$), from D1 to D10 ($p = 0.008$), and from D5 to D10 ($p = 0.008$). There was no interaction effect between day and sex on HR rest ($F_{(2, 16)} = 0.383$, $p = 0.688$, $np^2 = 0.046$).

Hsp 72 mRNA responses to heat acclimation between sexes

Figure 2 presents the means \pm SD for Hsp72 mRNA, pre and post on D1, D5, and D10 of controlled hyperthermia HA between males and females. Figure 2 also presents individual participants percentage change relative to D1. There was a main effect of time on Hsp72 mRNA response ($F_{(1,8)} = 32.998$, $p \leq 0.001$, $np^2 = 0.805$). Hsp 72 mRNA increased pre to post on D1 (1.7 ± 0.8 fold, 3.9 ± 1.8 fold; $p = 0.003$), D5 (1.6 ± 0.8 fold, 3.5 ± 1.7 fold; $p \leq 0.001$), and D10 (2.0 ± 0.7 fold, 3.0 ± 1.4 fold; $p = 0.050$). There was no interaction effect between time and sex ($F_{(2, 16)} = 1.027$, $p = 0.381$, $np^2 = 0.114$). The increase in Hsp72 mRNA from pre to post was similar between males (D1 = 1.8 ± 1.5 fold; D5 = 2.0 ± 1.0 fold; D10 = 1.1 ± 0.4 fold) and females (D1 = 2.6 ± 1.8 fold; D5 = 1.8 ± 1.4 fold; D10 = 0.9 ± 1.9 fold).

235 There was no main effect of day on Hsp72 mRNA ($F_{(1, 8)} = 0.052$, $p = 0.826$, $np^2 = 0.006$). There was no
236 interaction effect between day and sex on Hsp72 mRNA ($F_{(2, 16)} = 1.027$, $p = 0.381$, $np^2 = 0.114$). There
237 was no interaction effect between time, day and sex on Hsp72 mRNA ($F_{(2, 16)} = 0.479$, $p = 0.628$, $np^2 =$
238 0.057).

239 **Comparable heat acclimation sessions**

240 Table 3 presents the mean \pm SD for performance and physiological variables during D1, D5, and D10
241 of controlled hyperthermia HA for males and females. There was no main effect of day ($F_{(2, 16)} = 3.042$,
242 $p = 0.076$, $np^2 = 0.275$) or interaction effect between day and sex ($F_{(2, 16)} = 0.234$, $p = 0.794$, $np^2 = 0.028$)
243 on ΔT_{re} . There was no main effect of day ($F_{(2, 16)} = 2.536$, $p = 0.143$, $np^2 = 0.241$) or interaction effect
244 between day and sex ($F_{(2, 16)} = 1.880$, $p = 0.185$, $np^2 = 0.190$) on mean T_{re} . There was no main effect for
245 day ($F_{(2, 16)} = 0.488$, $p = 0.623$, $np^2 = 0.057$) or interaction effect between day and sex ($F_{(2, 16)} = 0.242$,
246 $p = 0.788$, $np^2 = 0.029$) on mean HR.

247 There was a main effect of day on relative exercise intensity ($F_{(2, 16)} = 21.593$, $p \leq 0.001$, $np^2 = 0.730$).
248 Relative exercise intensity was higher on D5 ($p \leq 0.001$) and D10 ($p \leq 0.001$) compared to D1. There
249 were no differences between D5 and D10 ($p = 0.221$). There was no interaction effect between day
250 and sex on relative exercise intensity ($F_{(2, 16)} = 0.1034$, $p = 0.378$, $np^2 = 0.114$).

251 **Table 3** Physiological and performance measures on D1, D5, and D10 of controlled hyperthermia heat acclimation. Mean ± SD.

	D1		D5		D10	
	Males	Female	Males	Females	Males	Females
T _{re} change (°C)	1.5 ± 0.2	1.5 ± 0.5	1.6 ± 0.4	1.40 ± 0.22	1.8 ± 0.3	1.8 ± 0.3
Mean T _{re} (°C)	38.2 ± 0.2	38.3 ± 0.2	38.1 ± 0.18	38.1 ± 0.1	38.2 ± 0.1	38.3 ± 0.9
Mean HR (beats.min ⁻¹)	146 ± 14	152 ± 9	145 ± 8	146 ± 10	143 ± 12	148 ± 9
Intensity (% $\dot{V}O_2$ peak)	38 ± 5	35 ± 8	50 ± 6	44 ± 7	52 ± 11	53 ± 14

252

253 **Notes:** HR, heart rate; T_{re}, rectal temperature; $\dot{V}O_2$ peak, peak oxygen uptake.

254 Discussion

255 This is the first study to compare Hsp72 mRNA expression in males and females across the course of
256 controlled hyperthermia HA. In contrast to our hypothesis, this experiment demonstrates that the
257 Hsp72 mRNA response is similar between males and females on D1, D5, and D10 of a controlled
258 hyperthermia HA. This suggests that sex differences in HSP following acute [20] and chronic [16] *in*
259 *vivo* exercise bouts are due to post transcriptional events. Controlled hyperthermia HA resulted in
260 typical phenotypic adaptations, evidenced by reduction in resting T_{re} and HR across the course of
261 controlled hyperthermia HA. Males and females demonstrated equal physiological responses (ΔT_{re} ,
262 mean T_{re} and HR) to each HA session where Hsp72 mRNA was measured. Accordingly, equality of these
263 endogenous stimuli, both between groups, and throughout HA elicited equal increases in Hsp72 mRNA
264 transcription. Comparable transcription of Hsp72 mRNA between males and females, suggests
265 endogenous stimuli which induce the HSR are the most important criteria for increasing Hsp72 mRNA
266 [7], with no sex dependent inhibition or amplification in transcription.

267 In the current study, HA produced a significant increase in Hsp72 mRNA providing further evidence
268 that the controlled hyperthermia HA method presents a sufficient endogenous stress to surpass the
269 minimum requirement to elicit increased transcription of Hsp72 mRNA, in both males and females, at
270 the onset and culmination of discrete and repeated bouts of exercise-heat stress. Gibson et al. [7]
271 reported an increase in Hsp72 mRNA pre to post on D1 (1.9 ± 0.6 fold, 4.9 ± 1.1 fold), D5 (2.3 ± 0.8
272 fold, 5.3 ± 2.5 fold) and D10 (2.1 ± 0.7 fold, 4.3 ± 1.3 fold) during a 10 d controlled hyperthermia HA
273 protocol in male participants. The data in the current study demonstrates females have a comparable
274 magnitude of response to males. Accordingly, this data provides mechanistic support for practitioners
275 prescribing controlled hyperthermia HA for female athletes. Sustained increases in Hsp72 mRNA
276 throughout the HA, further demonstrates the continued stimulation of the pathways responsible for
277 thermotolerance, i.e. the equality of HSR, in both males and females.

Previously, a greater HSP72 increase has been reported in males compared to females [16,20]; however, these studies measured the protein (HSP) whereas the current study measured the gene (Hsp mRNA). Interestingly, the current data contradicts Paroo et al. [29] findings, who reported a sex specific HSR at the level of protein and mRNA; with male rats having a significantly higher HSP70 (200% of control) and Hsp70 mRNA (+900% of control) response following 60 min of exercise at 70% $\dot{V}O_2$ max when compared with females (HSP70 = 100% of control; Hsp70 mRNA = 450% of control). Paroo et al. [29] did however provide mechanistic evidence for an interaction between HSP70, Hsp70 mRNA and oestrogen. Ovariectomized female animals treated with a placebo demonstrated equivalent increases in HSP72 (+150% of control) and Hsp72 mRNA (+1,200% of control) to males, whilst endogenous oestrogen returned the typical inhibited female HSR response (HSP70 100% of control; Hsp70 mRNA 300% of control). Methodologically, Paroo et al. [29] implemented the northern blotting technique which is less sensitive than the RT- QPCR technique used in the current study, potentially explaining the non-significantly increased Hsp72 mRNA.

Elevated oestrogen affords cellular protection [17] and thus, this cytoprotective pathway may inhibit changes in HSP72 translation [18]. It is likely, that oestrogen most greatly mediates post Hsp72 mRNA transcriptional changes which inhibit the translation of HSP72. The mechanism by which oestrogen attenuates HSR may be mediated through its indirect antioxidant properties by stabilising cellular membranes and attenuating oxidative stress; such an effect could protect thermal sensitive cells against exercise-induced damage, and thereby result in a blunted HSP72 response [29]. The lack of observed difference between males and females in the current study, may be a result of low oestrogen concentrations, which may not have been sufficient to exert an antioxidant effect.

Limitations

Future work should involve the measurement of HSP72 protein alongside Hsp72 mRNA across the course of controlled hyperthermia HA in males and females, to help underpin the true effect of sex on

the HSR; the absence of which is a limitation of the present study. Furthermore, oestrogen is reported to have a dose dependent inhibition of HSP72 expression at the transcription level [17]. Future work should investigate the HSR, and subsequent HSP72 and Hsp72 mRNA response to discrete and repeated bouts of exercise-heat stress in high and low oestrogen conditions and in post-menopausal women, who naturally have lower oestrogen concentrations. This information would help practitioners implement controlled hyperthermia HA strategies that ensure an optimal stimulus for cellular adaptation.

Conclusion

Males and females have equal Hsp72 mRNA expression throughout 10 d of controlled hyperthermia HA. This suggests that there are no differences in the endogenous criteria to transcribe Hsp72 mRNA via the HSR between males and females. Differences in basal HSP72 observed elsewhere are therefore likely to result from inhibited protein translation, potentially due to the influence of oestrogen.

322 References

- 323 [1] N.S. Taylor, *Human heat adaptation.*, Compr. Physiol. 4 (2014), pp. 325–65.
- 324 [2] M. Horowitz, *Heat acclimation, epigenetics, and cytoprotection memory.*,
325 Compr. Physiol. 4 (2014), pp. 199–230.
- 326 [3] M.N. Sawka, L.R. Leon, S.J. Montain and L.A. Sonna, *Integrated physiological*
327 *mechanisms of exercise performance , adaptation , and maladaptation to heat*
328 *stress*, Compr. Physiol. 1 (2011), pp. 1883–1928.
- 329 [4] J.D. Périard, S. Racinais and M.N. Sawka, *Adaptations and mechanisms of*
330 *human heat acclimation: Applications for competitive athletes and sports*,
331 Scand. J. Med. Sci. Sports 25 (2015), pp. 20–38.
- 332 [5] P.L. Moseley, *Heat shock proteins and heat adaptation of the whole organism*,
333 J. Appl. Physiol. 83 (1997), pp. 1413–1417.
- 334 [6] J.P. McClung, J.D. Hasday, J.R. He, S.J. Montain, S.N. Cheuvront, M.N. Sawka et
335 al., *Exercise-heat acclimation in humans alters baseline levels and ex vivo heat*
336 *inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells*, Am. J.
337 Physiol. Regul. Integr. Comp. Physiol. 294 (2008), pp. R185–191.
- 338 [7] O.R. Gibson, J.A. Mee, L. Taylor, J.A. Tuttle, P.W. Watt and N.S. Maxwell,
339 *Isothermic and fixed-intensity heat acclimation methods elicit equal increases*
340 *in Hsp72 mRNA*, Scand. J. Med. Sci. Sports 25 (2015), pp. 259–268.
- 341 [8] O.R. Gibson, G. Turner, J.A. Tuttle, L. Taylor, P.W. Watt and N.S. Maxwell, *Heat*
342 *Acclimation attenuates physiological strain and the Hsp72, but not Hsp90α*
343 *mRNA response to acute normobaric hypoxia.*, J. Appl. Physiol. 119 (2015), pp.
344 889–899.
- 345 [9] B.J. Lee, A. Miller, R.S. James and C.D. Thake, *Cross acclimation between heat*
346 *and hypoxia: Heat acclimation improves cellular tolerance and exercise*
347 *performance in acute normobaric hypoxia*, Front. Physiol. 7 (2016), pp. 1–15.
- 348 [10] S.M. Senf, T.M. Howard, B. Ahn, L.F. Ferreira and A.R. Judge, *Loss of the*
349 *Inducible Hsp70 Delays the Inflammatory Response to Skeletal Muscle Injury*
350 *and Severely Impairs Muscle Regeneration*, PLoS One 8 (2013), .
- 351 [11] B.G. Drew, V. Ribas, J.A. Le, D.C. Henstridge, J. Phun, Z. Zhou et al., *HSP72 is a*
352 *mitochondrial stress sensor critical for Parkin action, oxidative metabolism, and*
353 *insulin sensitivity in skeletal muscle*, Diabetes 63 (2014), pp. 1488–1505.
- 354 [12] S.H. Lee, H.M. Kwon, Y.J. Kim, K.M. Lee, M. Kim and B.W. Yoon, *Effects of*
355 *Hsp70.1 gene knockout on the mitochondrial apoptotic pathway after focal*
356 *cerebral ischemia*, Stroke 35 (2004), pp. 2195–2199.
- 357 [13] M. Kuennen, T. Gillum, K. Dokladny, E. Bedrick and S. Schneider,
358 *Thermotolerance and heat acclimation may share a common mechanism in*

- 359 *humans*, Am. J. Physiol. Regul. Integr. Comp. Physiol. 1 (2011), pp. 524–533.
- 360 [14] D.S. Moran, L. Eli-Berchoer, Y. Heled, L. Mendel, M. Schocina and M. Horowitz,
361 *Heat intolerance: does gene transcription contribute?*, J. Appl. Physiol. 100
362 (2006), pp. 1370–1376.
- 363 [15] J.T. Silver and E.G. Noble, *Regulation of survival gene hsp70.*, Cell Stress
364 Chaperones 17 (2012), pp. 1–9.
- 365 [16] J.P. Morton, K. Holloway, P. Woods, N.T. Cable, J. Burniston, L. Evans et al.,
366 *Exercise training-induced gender-specific heat shock protein adaptations in*
367 *human skeletal muscle.*, Muscle Nerve 39 (2009), pp. 230–233.
- 368 [17] T. Shinohara, N. Takahashi, T. Ooie, M. Ichinose, M. Hara, H. Yonemochi et al.,
369 *Estrogen inhibits hyperthermia-induced expression of heat-shock protein 72*
370 *and cardioprotection against ischemia/reperfusion injury in female rat heart.*, J.
371 Mol. Cell. Cardiol. 37 (2004), pp. 1053–1061.
- 372 [18] E. Bombardier, C. Vigna, S. Iqbal, P.M. Tiidus and A.R. Tupling, *Effects of*
373 *ovarian sex hormones and downhill running on fiber-type-specific HSP70*
374 *expression in rat soleus.*, J. Appl. Physiol. 106 (2009), pp. 2009–15.
- 375 [19] H. Ogita, K. Node and M. Kitakaze, *The role of estrogen and estrogen-related*
376 *drugs in cardiovascular diseases*, Curr. Drug Metab. 4 (2003), pp. 497–504.
- 377 [20] T. Gillum, M. Kuennen, C. Gourley, K. Dokladny, S. Schneider and P. Moseley,
378 *Sex differences in heat shock protein 72 expression in peripheral blood*
379 *mononuclear cells to acute exercise in the heat.*, Int. J. Endocrinol. Metab. 11
380 (2013), pp. 8739.
- 381 [21] J.A. Mee, O.R. Gibson, J. Doust and N.S. Maxwell, *A comparison of males and*
382 *females' temporal patterning to short- and long-term heat acclimation*, Scand.
383 J. Med. Sci. Sports 25 (2015), pp. 250–258.
- 384 [22] C.M. Winget, C.W. DeRoshia and D.C. Holey, *Circadian rhythms and athletic*
385 *performance*, Med. Sci. Sport Exerc. 17 (1985), pp. 498–516.
- 386 [23] L. Taylor, A.W. Midgley, B. Christmas, A.R. Hilman, L. a Madden, R. V Vince et
387 al., *Daily hypoxia increases basal monocyte HSP72 expression in healthy human*
388 *subjects.*, Amino Acids 40 (2011), pp. 393–401.
- 389 [24] M.N. Sawka, L.M. Burke, E.R. Eichner, R.J. Maughan, S.J. Montain and N.S.
390 Stachenfeld, *American College of Sports Medicine position stand. Exercise and*
391 *fluid replacement.*, Med. Sci. Sport Exerc. 39 (2007), pp. 377–390.
- 392 [25] R.H. Fox, R. Goldsmith, D.J. Kidd and H.E. Lewis, *Acclimatization to heat in man*
393 *by controlled elevation of body temperature*, J. Physiol. 166 (1963), pp. 530–
394 547.
- 395 [26] O.R. Gibson, J.A. Mee, J.A. Tuttle, L. Taylor, P.W. Watt and N.S. Maxwell,

396 *Isothermic and fixed intensity heat acclimation methods induce similar heat*
397 *adaptation following short and long-term timescales*, J. Therm. Biol. 49–50
398 (2015), pp. 55–65.

399 [27] P. Chomczynski and N. Sacchi, *Single-step method of RNA isolation by acid*
400 *guanidinium thiocyanate-phenol-chloroform extraction*, Anal. Biochem. 162
401 (1987), pp. 156–159.

402 [28] T.D. Schmittgen and K.J. Livak, *Analyzing real-time PCR data by the*
403 *comparative CT method*, Nat. Protoc. 3 (2008), pp. 1101–1108.

404 [29] Z. Paroo, E.S. Dipchand and E.G. Noble, *Estrogen attenuates postexercise*
405 *HSP70 expression in skeletal muscle.*, Am. J. Physiol. Cell Physiol. 282 (2002),
406 pp. C245–51.

407